IN THE SPECIFICATION:

Paragraph bridging pages 1 and 2.

In the calf which is exposed for the first time, the disease (dictyocaulosis) is caused by ingestion of the third larvae together with the pasture grass. By way of the blood system, the larvae reach the alveoli of the lungs, which they penetrate in order to reach the air-conducting parts of the lung. During this process, lesions are produced which serve as the port of entry for secondary bacterial infections; the multiplication of bacteria and other microbial pathogens leads to limited or generalized lung inflammations with all the possible sequelae such as pulmonary edema and heart failure (T. Schneider, A. Bellmer, F.-J. Kaup (1989) Wien. Tierärztl. Mschr. 76:372-476). Breathing is also substantially impeded by the adult stages, which are present in the upper airways and which lead to obstructions. Visible consequences of the marked impairment of general well-being are reduced weight increases, or even weight losses, which are associated with growth delays. From time to time, the clinical symptoms worsen dramatically and rapidly lead to death. Lungworm disease in cattle can be diagnosed on the basis of the clinical symptoms (G. Gräfner (1987) Monatsh. Vet. med. 42: 178-181) or on the basis of the larvae which are egested with the feces (J. Boch, R. Supperer (1992)). These possibilities are especially suitable for diagnosing the disease in the individual animal which is heavily infected. However, modern large-scale livestock farming requires epidemiological predictions and risk assessments with regard to the possibility of an outbreak of dictyocaulosis in the late pasture season, with these predictions and assessments being based on suitable diagnosis; i.e. many, possibly still only lightly infected calves have to be investigated in surveys using a safe and sensitive method. Serological methods are suitable for this purpose (A. Bellmer, T. Schnieder, A.M. Tenter (1989) Proc. 13th Conf. Wrld

Ass. Adv. Vet. Parasit., pp. 33, Berlin, 07.-11.08.1989). Antigens which are identified in Dictyocaulus viviparus and then isolated, and in some cases prepared recombinantly, are used for the serodiagnosis. Medicaments which are active against adult and juvenile stages (e.g. LEVAMISOL®, (pro) benzimidazoles, NETOMIN® or IVERMECTIN®) can be employed for treating the dictycaulosis. These preparations are highly effective and are consequently usually able to prevent losses due to acute lungworm diseases (H. Mehlhorn, D. Düwel, W. Raether (1993) Diagnose und Therapie der Parasitosen von Haus-, Nutz-und Heimtieren (Diagnosis and Therapy of the Parasitoses of Domestic and Economically Useful Animals). 2nd ed. Gustav Fischer Verlag, pp. 223-227). Because of their drastic effect, the active compounds may, in association with a prophylactic/metaphylactic treatment, possibly not allow the parasite to interact with the immune system of the host and consequently not allow a resilient (partial) immunity to develop and be maintained. The animals are then exposed, in an unprotected state, to an infection in the second year at pasture (COBS, D.E., S.R. Pitt, J. Förster, M.T. Fox (1987) Res. Vet. Sci. 43:273-275).

D1 Cont

Page 3, second paragraph

The invention relates to a novel, immunogenic native protein, termed DV 17, which was isolated from adult Dictyocaulus viviparus worms. Its immunogenicity is based, in particular, on the fact that after it has been administered subcutaneously to cattle, it induces an antibody response which confers immunoprotection on the animal. In addition, this protein can be used in an ELISA for retrospectively immunodiagnosing dictyocaulosis in cattle. DV 17 is characterized by the following physical properties. The protein is stable in all buffers employed. No decrease in immunoreactivity was observed after the purified antigen had been deep frozen (-85°C). Using an HPLC system and a NUCLEOSIL™ C 18 column (150 mm x 4.6 mm; 5

D2

u), the retention time for antigen DV 17 was measured to be 14 min (gradient elution comprising distilled water/0.1% TFA (=0%B) and acetonitrile/0.1% TFA (=100% B)). DV 17 has an estimated molecular weight of approx. 16,500 daltons in SDS-polyacrylamide gel electrophoresis (PHASTGEL™ 8-25%). The isoelectric point of DV 17 is in the range of 5.3-5.9. Finally, the part amino acid sequences depicted in Table 1 were determined following proteolysis with endopeptidase Lys C.

D2 Cont

Page 7, second paragraph

Chromatographically separated protein fractions obtained from homogenized adult lungworms were subjected to further fractionation by means of SDS polyacrylamide gel electrophoresis and immobilized on IMMOBILON™ P membranes (semidry blotting). The lungworm-specific protein DV 17 was then detected using the specific sera from infected animals and then purified further using a reverse phase HPLC column. The purity of the protein fraction was checked in silver-stained SDS polyacrylamide gels (PHASTGELS™). A BCA protein assay was used to determine the protein concentration in electroprorectically pure DV 17 fractions and the latter were then deep frozen at -85°C.

D3

Helminth-naive cattle were vaccinated 2 times with a defined quantity of purified DV 17 on each occasion. One week after the second vaccination, the cattle were challenged with Dictyocaulus viviparus L3 larvae. Unvaccinated animals served as controls. 4 weeks after the challenge, the cattle were slaughtered and the number of adult worms in the lung was determined and the lengths of the male and female worms were measured. The reduction in the number of adult worms as compared with the unvaccinated control was defined as the measure of the immunoprotection.

Page 8, third paragraph - page 9

D4

10 g of frozen worm mass were thawed at room temperature and then

homogenized together with 40 ml of a 0.025 M solution of Tris-HCl, pH 7.4, containing 2 mM PEFABLOC®, in a tissue homogenizer. In order to remove coarse tissue constituents, the homogenate was centrifuged at 3010 g for 15 min at 4°C and the pellet was discarded. The supernatant was centrifuged at 39,800 g for 20 min at 4°C and the supernatant from this centrifugation was then recentrifuged under the same conditions for 10 min. Following filtration using 1.2 µm filters, the clear supernatant was dialyzed (cut-off of the dialysis membrane, 8000) at 4°C overnight in 1 liter of phosphate-buffered sodium chloride solution (PBS).

D4 cont

Page 9, Second paragraph

The dialyzed supernatant was centrifuged at 39,800 g for 15 min and the clear supernatant was fractionated in a Pharmacia FPLC system using a preparative gel filtration column (column type XK 16/60; separation medium: SUPERDEX™ 75 prep grade, column volume: 124 ml). PBS, pH 7.4, was used for the elution. The fractions having the retention volumes 65-75 ml were collected and concentrated using ultrafiltration modules (cut-off 3000). Protein DV 17 was detected using an amplified Western blot.

D5

Page 9, Third paragraph

The concentrated SUPERDEX™ 75 prep grade fraction was mixed with reduced SDS buffer in a ratio of 1:2; 40 µl of this mixture were added to each well on an SDS-EXCEL™ gel (from Pharmacia). The electrophoresis was carried out in a MULTIPHOR™ II chamber (from Pharmacia) under standardized running conditions (600 V, 50 mA, 30 W, running time: 90 min). The electrophoretically separated proteins were transferred onto IMMOBILON P™ membranes by means of semidry blotting (Tovey ER, Baldo BA, Electrophoresis. 8, 1987, 384-387) (transfer conditions: 45 min, constant current strength of 0.8 mA/cm^2) and, after a 24-hour blocking phase using 3%

D6

bovine serum albumin in Tris-buffered sodium chloride solution (TBS), incubated for 1 hour with a Dictyocaulus-specific immune serum (obtained on D+40, see Example 1) used at a dilution of 1:20. Normal bovine serum (dilution 1:20) was used as the negative control. After having been washed 3 times with TBS + 0.05% TWEEN 20™, the blot membrane was incubated for 1 hour with a biotin-labeled goat anti-bovine IgG (H+L) antibody (1:500; from Pierce). After having been washed (TBS + 0.05% TWEEN 20™) 3 times, the membrane was incubated for 1 hour with a biotin/streptavidin/alkaline phosphatase enzyme conjugate (1:2500; from Pierce). The substrate was developed using the substrate kit supplied by Biorad.

D6 Cont

Page 10, second paragraph

After the DV 17 had been immunologically identified in the preparative gel filtration fractions, the protein was purified further by means of HPLC. A NUCLEOSIL™ C 18 5U column from Alltech (150 mm x 4.6 mm) was used for this purpose. The protein was eluted with a linear buffer gradient (buffer A: high-purity water +0.1% trifluoroacetic acid (TFA); buffer B: acetonitrile + 0.1% TFA). 500 µl of the fraction which was concentrated in Example 4 were diluted 1:2 with buffer A and then injected into the column. The flow rate was 0.5 ml/min. The gradient elution was started 5 min after the injection and terminated 10 min later. DV 17 was found to have a retention time of 14 min.

D7

Page 10, Third paragraph

The HPLC-purified fraction having a retention time of 14 min was analyzed on a PHAST™ system (Pharmacia) SDS polyacrylamide gel (8-25% PHAST™ SDS gel) under standardized conditions. The "Silver Stain SDS-PAGE Standards, low range" kit supplied by Biorad was used for the molecular weight markers. After the electrophoresis, DV 17 was visualized by silver staining

D8

(Pharmacia Silverstain kit). The molecular weight was determined with a videodensitometer (Biorad Molecular Analyst) using the "Profile analyst II" evaluation program. DV 17 was calculated to have a molecular weight of 16,500 ± 1500 daltons.

D8 Cont

Page 11, first paragraph

DV 17 which had been isolated as described in Example 6 was diluted with high-purity water and loaded onto previously prepared focusing gels (pH 3-10 IEF PHAST™ gels, from Pharmacia). The focusing in the PHAST™ system was carried out under standardized conditions. Marker proteins having defined isoelectric points (pH 3.5-9.3, from Pharmacia) were included for the purpose of determining the isoelectric point of DV 17. The latter was found to have an isoelectric point of 5.3-5.9.

D9

Page 12, first paragraph

ELISA plates, (MAXISORB™ from Nunc) were coated with a concentration of 5 µg of DV 17/ml of PBS; the reaction volume was 100 µl per well. After having been incubated at 37 °C for 1 hour, the plates were washed 3 times in an ELISA washer, with the wash volume being 200 µl per well. The washing solution used was high-purity water +0.1% TWEEN 20™. Nonspecific binding sites were blocked by incubating (3 hours at room temperature) with a proteolytic mixture of gelatin (from Boehringer Mannheim). The incubation took place on a microtiter plate shaker at a shaking frequency of 300 rpm. After the plates have been washed 3 times, the wells were loaded with a 1:200 dilution of specific infection sera and control sera and incubated at room temperature for 1 hour. After the plates had been washed 3 times, peroxidase-conjugated, polyclonal rabbit antibody against bovine IgG (Fc fragment-specific; from Dianova) was used, at a dilution of 1:10,000, as the detection antibody; the duration of the incubation was 30 min. The plates were then washed 4 times and

D10

incubated with substrate solution (composition: 5 ml of 10 times concentrated ABTS buffer + 45 ml of high-purity water + 1 tablet of ABTS (about 50 mg of ABTS). Substrate development took place at room temperature and was monitored every 10 min in an ELISA rader at 414 nm. In the ELISA, it was possible to detect Dictyocaulus-specific antibodies 20 days after a lungworm infection at the earliest.

D10 Cont

Page 12, Second paragraph

"DYNABEADS™ "(DYNABEADS™ mRNAdirect hit, DYNAL) were used to isolate total D. viviparus RNA. 400 ng of this RNA was precipitated with ethanol and used as the template for a RACE (reamplification of cDNA ends; Frohmann et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:8998-9002) cDNA synthesis, employing the "Marathon cDNA Amplification Kit" (Clontech). The cDNA adapter-specific primers from the said kit, and the "Expand Long Template PCR System" (Boehringer Mannheim) were used. Conditions: 600 nM of each primer; 1.75 mM $MgCl_2$, 400 mM dNTPs, 392 ng of Taq Start Antibody (Clontech) and 0.35 U of DNA polymerase mix. The temperature profile was as follows:

D11

Cycle 1	92°C	120 s
	60°C	60 s
	68°C	360 s
Cycles 2-30	92°C	60 s
	60°C	60 s
	68°C	360 s

Page 13, third full paragraph

D12

Five DNA fragments were obtained after 2 rounds of PCT in a PE 9600™ Thermal Cylcer (Perkin Elmer) (500 nM of each primer; 0.1 vol of PCR buffer; 200 mM dNTPs; 1.25 units of AMPLI TAQ GOLD™ (Perkin Elmer). The following temperature profile was used:

Cycle 1	92°C	600 s
Cycles 2-31	94°C	40 s
	55°C	40 s
	72°C	60 s

D2 cont

Page 14, second full paragraph

D3 The RACE reaction mixtures contained 600 nM concentrations of each primer; 0.2µl of diluted, amplified DV 17 RACE cDNA; 0.1 vol of PCR buffer I; 200 µM dNTPs and 1.25 U of AMPLI TAQ GOLD™. The temperature profile was the same as in the PCR reaction using the non-degenerate primers apart from the fact that the annealing temperature was 60°C and the samples were left for 360 s at 72°C in the last cycle.